Canaigre Investigations*

V. Analytical Studies on the Extraction of Canaigre Roots with Water and with Acetone-Water Mixtures†

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In investigations of canaigre, *Rumex hymenosepalus* Torr, as a source of tannin it has been necessary to analyze many samples of canaigre root. The high starch content of the roots makes the complete extraction of tannin difficult. In discussing the use of several tannin extractors, Veitch[11] stated that because of starch the canaigre could not be extracted efficiently above 50° C. Procter[7] showed that the optimum extraction temperature is between 50° and 60° C. Beebe, Cordon and Rogers[2], in their centrifugal leaching of canaigre, found that leaching efficiencies were in some cases above 100 per cent. They attributed these results in part to incomplete recovery of tannin by present analytical extraction methods.

In the analyses of roots grown on experimental plots, it has been difficult to obtain concordant results. This difficulty has been attributed to the use of coarse samples and to incomplete extraction of tannin. Since the starch made satisfactory extraction of finely ground samples with water impossible, the fresh canaigre roots were prepared for extraction by slicing in thin slices in a commercial potato slicer, and air drying. The extraction was conducted in a Reed-Churchill extractor[4] at 65° C., one liter of extract being collected in 7 hours. There was no apparent clogging of the extractor under these conditions. Partial gelatinization of starch, however, occurred at this temperature, and water solubles were withheld. One liter of water passing over the sample was not sufficient to break this occlusion and give complete extraction of tannin.

In order to appraise accurately the efficiency of a commercial leaching or extraction process for tannin, it is essential that the tannin content of the raw material be known. The studies reported here were undertaken with the object of developing a more efficient method for the extraction of tannin from canaigre. They dealt with the effects of particle size of the sample, volume of extractive, temperature of extraction, and the use of water and acetone-water mixtures as solvents.

†This is the fifth of a series of papers reporting various phases of cooperative investigations of canaigre as a source of tannin by the Bureau of Agricultural and Industrial Chemistry and the Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture and the Texas Agricultural Experiment Station. [8,9,3]

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Experimental Procedures and Results

In order to illustrate the lack of concordance obtained in different extractions, analyses of solutions from five replicate extractions are given in Table I. The sample used was typical of those that failed to give good duplicate results on analysis.

TABLE I

Variations in Analyses of One Sample of Sliced Canaigre Roots Extracted with Water*

(Results on Moisture-Free Basis)

Analysis Number	Total Extractives	Soluble Extractives	Insolubles	Non Tannin	Tannin	Purity†
	%	%	%	%	%	
1	43.7	42.4	1.3	19.4	23.0	54.2
2	41.5	40.1	1.4	18.7	21.4	53.4
3	39.5	38.2	1.3	17.9	20.3	53.1
4	42.6	41.2	1.4	19.4	21.8	52.9
5	44.5	42.8	1.7	19.4	23.4	54.7

*Extracted in a Reed-Churchill extractor at 65° C.
†Purity is 100 times the tannin divided by the soluble extractives.

This particular sample of canaigre was harvested at State College, New Mexico, in 1945 and was from a plot in which fertilizer requirement studies were being conducted. The data show large variations in the percentages of tannin and soluble extractives; the values for non tannin, with one exception, show good agreement. These data, together with the past experience of the authors, indicate that tannins are not so easily extracted as non tannins. They are probably withheld by the gelatinized starch, which may account for poor agreement between duplicate analyses.

Water Extractions

Effect of Particle Size:

Although thinly sliced canaigre roots can be extracted in a Reed-Churchill extractor with water at 65° C. without clogging the extraction tube, such a coarse sample is not well adapted to the most efficient extraction of tannin, and this might explain in part the lack of concordance of results and the low tannin values obtained. To obtain information on this point, some preliminary studies of the effect of particle size of sample were undertaken.

For this work it was not feasible to employ the regular Reed-Churchill extractor at 65° C. because when finely ground samples were used the starch became partially gelatinized and caused clogging.

A special extraction apparatus was used for these studies. It consisted of a boiling flask connected to a trap, which in turn was connected to a condenser. When the apparatus is in operation, the condensed water runs back

into the trap and is kept near the boiling point by the steam from the generator. This hot water runs to a bulb, which serves as a cooling chamber. From here the cooled water is led to the extraction tube, which contains the sample. The cooling bulb was so constructed that the water could be chilled to any desired temperature. This equipment, which for convenience has been designated as an "outside extractor," made possible the removal of the first portions of extract without boiling, and also the continued refluxing of the sample at 60° to 62° C. for any desired period. Slices from the same sample were extracted in the Reed-Churchill extractor for comparison.

The sample used was harvested at Sacaton, Arizona, sliced in a commercial potato slicer, mixed, and divided into three equal portions. One was retained in the form of slices to be used as a control, and the other two were ground in a Wiley mill to pass 4 mm. and 2 mm. screens, respectively.

Preliminary data showing the effect of particle size in the extraction of canaigre with water are given in Table II.

TABLE II

EFFECT OF PARTICLE SIZE ON THE EXTRACTION OF CANAIGRE WITH WATER

(Results on Moisture-Free Basis)*

Particle Size	Total Extractives	Soluble Extractives	Insolubles	Non Tannin	Tannin	± Deviation	Purity†
	%	%	%	%	%		
			Outside Extractor at 60° to 62° C.				
Slices‡	57.1	54.3	2.8	22.8	31.5	±0.3	58.0
4 mm.	59.2	55.9	3.3	23.2	32.7	±0.2	58.5
2 mm.	58.5	54.5	4.0	22.7	31.8	±0.2	58.3
			Reed-Churchill Extractor at 65° C.				
Slices‡	55.2	53.8	1.4	22.7	31.1	±0.1	57.8

*All results are averages of duplicate analyses.
†Purity is 100 times the tannin value divided by soluble extractives.
‡Roots were sliced in a potato-slicing machine, which gave fairly uniform thin slices.

There was little difference in the extractives of the sliced material obtained by the use of the two different types of extractors, although the outside extractor passed a much greater volume of water through the sample. The finer and more uniform preparation, ground to pass a 4 mm. screen, gave a more complete extraction of tannin, showing 1.2 per cent higher tannin than was obtained from the sliced sample. The portion of sample ground to pass a 2 mm. screen could be extracted only after mixing with purified quartz sand, and even with this treatment gave an inefficient extraction, apparently owing to the large amounts of fines or the swelling of starch.

Several unreported experiments conducted for the comparison of the outside and Reed-Churchill extractors have shown that the outside extractor

gave tannin values which ranged from 0.4 to 2.1 per cent higher than those obtained with the Reed-Churchill extractor. In general, the non tannins remained constant, and the increase in tannin was obtained as the result of more complete extraction, as is shown in Table II.

Effects of Volume of Water and Temperature:

The effects of volume of water passing through the sample, and the temperature of extraction were investigated. In order to make a comparison test of all extractors, slices were used which would permit percolation in all cases. Two samples of sliced, dried canaigre roots were each extracted at 45°, 50°, 55°, 60°, and 65° C. in a Reed-Churchill extractor, one and two liters of extractives, respectively, being collected in 7 hours. Extractions were made in triplicate. In the two-liter collections, the amount of wet chromed hide powder used for detannization was reduced proportionately. In all other respects, the extracts were analyzed for tannin by the Official methods of the American Leather Chemists Association.[1] Extractions at the lower temperatures were made to avoid starch gelatinization. It was thought that doubling the volume of water used might increase the amount of tannin extracted. The results of these studies are summarized in Table III.

It will be noted that, with one exception, the highest tannin values obtained by extraction in the Reed-Churchill extractors were obtained at 60° and 65° C. Contrary to expectations, doubling the volume of extractives gave slightly lower tannin values in all cases except one. This probably resulted from the reduction in the amount of hide powder used.*

In Table III average results are given for comparable extractions of these samples with the outside, Reed-Churchill and Clarke-Frey extractors. The Official A.L.C.A. reflux method and the Clarke-Frey extractor gave the highest tannin and total extractive values but also gave high percentages of insolubles. Moreover, the extractives filtered so slowly during analysis that the aliquots required for the soluble solids and non tannin determinations could not be obtained in a reasonable time. This method, therefore, is not a practical procedure for the regular analysis of canaigre.

The outside extractor had the advantage of the low extraction temperature of 60° to 62° C. and permitted the passage of a large volume of water through the sample as was done in the reflux method. Except for the lower temperature, the extraction with the outside extractor was made according to the reflux method. The values obtained with the outside extractor were slightly higher than those with the Reed-Churchill extractor, but lower than those with the reflux method. In order to obtain the maximum amount of extractives from sliced canaigre roots with water, it appears that higher temperatures and

* Laboratory tests have shown that if an analytical tannin solution showing 4 g. of tannin per liter by the Official A.L.C.A. method, in which 46 g. of chromed hide powder is used per 200 ml. of solution, is diluted to double its volume and a 200 ml. aliquot is detannized with 23 g. of chromed hide powder, the value obtained for percentage tannin is lower than that found by the Official method.

TABLE III

EFFECT OF TEMPERATURE AND VOLUME OF EXTRACTIVES ON THE ANALYSES OF SLICED CANAIGRE ROOTS EXTRACTED WITH WATER

(Results on Moisture-Free Basis)

Temperature	Volume of Extractives	Sample A						Sample B					
		Total Extractives	Soluble Extractives	Insolubles	Non Tannin	Tannin	Purity†	Total Extractives	Soluble Extractives	Insolubles	Non Tannin	Tannin	Purity†
°C.	liters	%	%	%	%	%		%	%	%	%	%	
						*Reed-Churchill Extractor**							
65	1	48.7	47.4	1.3	22.3	25.1	53.0	47.5	46.5	1.0	22.6	23.9	51.4
65	2	49.8	48.0	1.8	23.1	24.9	51.9	48.6	47.5	1.1	24.2	23.3	49.1
60	1	49.1	47.6	1.5	22.3	25.3	53.2	47.0	46.0	1.0	22.6	23.4	50.9
60	2	50.3	48.5	1.8	23.5	25.0	51.5	47.5	46.3	1.2	23.9	22.4	48.4
55	1	47.0	46.2	0.8	22.1	24.1	52.2	47.5	46.7	0.8	23.2	23.5	50.3
55	2	48.2	47.5	0.7	23.3	24.2	50.9	47.7	46.9	0.8	23.9	23.0	49.0
50	1	47.3	46.8	0.5	22.3	24.5	52.4	46.9	46.0	0.9	23.0	23.0	50.0
50	2	48.1	47.2	0.9	23.3	23.9	50.6	46.4	46.0	0.4	23.8	22.2	48.3
45	1	47.2	46.9	0.3	22.3	24.6	52.4	44.9	44.7	0.2	22.6	22.1	49.4
45	2	47.2	46.8	0.4	22.9	23.9	51.1	45.5	45.1	0.4	23.8	21.3	47.2
						Reflux Method—Clarke-Frey Extraction Tube‡							
92	1	63.0	54.7	8.3	26.3	28.4	51.9	60.5	53.0	7.5	26.0	27.0	50.9
						Reflux Method—Outside Extractor‡							
60-62	1	50.5	48.9	1.6	22.6	26.3	53.8	50.0	48.4	1.6	23.3	25.1	51.8

*Results are the average of triplicate analyses.

†Purity is 100 times the tannin value divided by soluble extractives.

‡Results are the average of duplicate analyses.

prolonged washing are necessary. This procedure, however, is not feasible with the present type of extractors, in which water is used as the solvent.

Acetone-Water Extractions

Almost all research on the extraction of vegetable tanning materials has involved the use of water as a solvent, and very little has been done on other solvents. Grassmann and Kuntara[5] extracted pine, larch and oak barks with organic solvents such as methanol, ethanol and acetone. The extracted barks were again extracted with water, and the yield of tannin from the combined extracts was considerably more than that given by the conventional water extraction. They also obtained good results with water-solvent mixtures. Stather, Lauffmann and Bau Miau,[10] who investigated the solubilities of vegetable tannin extracts in organic solvents and water, have shown that the solubility of vegetable tannin extracts in a mixture of water with 25 to 50 per cent of methanol, ethanol or acetone is greater than in pure water.

In view of these findings, it was considered reasonable to expect higher tannin yields from canaigre with similar solvents. Acetone seemed a logical choice because of its low-boiling point and nongelatinizing action on starch. Preliminary tests with acetone-water mixtures gave promising results.

Preliminary Tests:

Extractions of canaigre were conducted at 60° C. by mixing with acetone-water mixtures containing, respectively 25, 50 and 75 per cent acetone by volume, followed by decantation and filtration. The sample had been passed through a 60 mesh screen. Extraction of a sample of this particle size was possible because the acetone-water solvent did not cause swelling of starch, with resultant clogging. Some difficulty was experienced, however, with the 25 per cent acetone-water mixture, perhaps because of partial starch gelatinization. The tannin extracted by this procedure was 4 to 5 per cent higher than that obtained by the water-extraction method usually employed. An extraction with a small laboratory-type basket centrifuge, in which the sample was subjected to four washings with a 50 per cent acetone-water mixture at 60° C., gave approximately the same results as were obtained by the decantation and filtration method. A similar extraction of the same sample was made in a Reed-Churchill extractor at 60° C. with a 50 per cent acetone-water mixture.

Table IV gives the results of the extractions with water alone and with acetone-water mixtures. The acetone-water mixtures gave much higher values for tannin and good agreement between the decantation and filtration, centrifuge and Reed-Churchill extraction methods. Again the increase in soluble extractives was largely due to tannin, indicating less occlusion of tannin by starch and therefore a more efficient extraction. The effect of the solvent action of pure acetone was studied by using a Soxhlet extractor for 18

TABLE IV

COMPARISON OF TANNIN VALUES OF CANAIGRE EXTRACTED WITH WATER AND WITH ACETONE-WATER MIXTURES

(Results on Moisture-Free Basis)

Type of Extraction	Temperature of Extraction	Solvent	Particle Size	Total Extractives	Soluble Extractives	Insolubles	Non Tannin	Tannin	Purity*
	°C.			%	%	%	%	%	
Reed-Churchill	65	water	slices	59.4	58.4	1.0	32.9	25.5	43.7
Outside	60–62	water	slices	60.8	59.5	1.3	33.5	26.0	43.7
Outside	60–62	water	4 mm.	62.2	60.7	1.5	33.7	27.0	44.5
Clarke-Frey	92	water	slices	70.4	62.2	8.2	35.9	26.3	42.3
Decantation and Filtration	55–60	25% acetone-water	60 mesh	63.2	61.6	1.6	32.4	29.2	47.4
Decantation and Filtration	55–60	50% acetone-water	60 mesh	63.8	62.0	1.8	32.0	30.0	48.4
Decantation and Filtration	55–60	75% acetone-water	60 mesh	64.0	62.1	1.9	31.9	30.2	48.6
Centrifuge	58–60	50% acetone-water	60 mesh	64.4	61.7	2.7	31.9	29.8	48.3
Reed-Churchill	60	50% acetone-water	60 mesh	63.0	61.2	1.8	32.0	29.2	47.7
Soxhlet		75% acetone-water	60 mesh	32.3	31.5	0.8	7.0	24.5	77.8

*Purity is 100 times the tannin divided by the soluble extractives.

hours and a 75 per cent acetone-water mixture, since this mixture gave an essentially pure acetone extraction liquid and yet provided a water solution of the extractives. The extractives in this case were lower than those obtained by other methods and extraction mixtures.

Before analysis, the acetone from all acetone-water extractives was removed by distillation. The residual aqueous solutions were transferred to one-liter volumetric flasks, diluted to volume and analyzed for tannin in the usual way. No difficulty was experienced in the filtration, and in these preliminary and roughly quantitative analyses good agreement between duplicates was obtained.

Further preliminary tests showed that samples of canaigre roots powdered by grinding in a hammer mill could be successfully extracted in a Reed-Churchill extractor at 60° C. with a 50 per cent acetone-water mixture. A sample of shredded canaigre root from New Mexico and one from Arizona when extracted with water gave tannin values of 20.2 and 26.0 per cent. The same samples when powdered in a hammer mill and extracted in a Reed-Churchill extractor with a 50 per cent acetone-water mixture gave tannin values of 27.8 and 33.2 per cent, respectively.

Effect of Particle Size:

After these preliminary tests, further extraction studies were conducted. Water and an acetone-water mixture were used for the extraction of one sample prepared in eight different states of subdivision. The canaigre used came from a bulk lot of roots harvested at State College, New Mexico in 1945. It had been cut in a shredding machine[6] and dried in a continuous commercial dryer. A representative sample was taken. It was mixed and subdivided by quartering into eight equal portions. One portion was left shredded and one was rolled with a glass jar in order to break the shreds into smaller, uniform particles without forming fines. Four portions were ground in a Wiley mill to pass 6.72 mm., 4 mm., 2 mm., and 1 mm. screens, respectively. The other two portions were ground in a mortar to pass 60 mesh (0.25 mm.) and 200 mesh (0.074 mm.) screens.

For each test, the quantity of material needed to give 4 grams of tannin per liter of solution was weighed into an extraction tube. Water extractions were conducted (a) in a Reed-Churchill extractor at 65° C. for seven hours and (b) in the outside extractor at 60° to 62° C. with refluxing up to 16 hours. At the end of the extraction period, the extractives were made to volume. Tannin was determined by the Official A.L.C.A. method.

In the mixed solvent extractions, the extraction tubes were placed in a Reed-Churchill extractor, which acted as a constant temperature bath, and maintained at the desired temperature of 60° C. throughout the extraction period. The 50 per cent acetone-water mixture at room temperature was added at regular intervals and allowed to percolate through the sample.

TABLE V

EFFECT OF PARTICLE SIZE ON THE EXTRACTION OF CANAIGRE WITH WATER AND WITH ACETONE-WATER MIXTURE

(Results on Moisture-Free Basis)

Particle Size	Total Extractives	Soluble Extractives	Insolubles	Non Tannin	Tannin	Purity*	Reducing Sugar	Total Sugar	pH of Soluble Extractives
	%	%	%	%	%		%	%	
Water Extraction—Reed-Churchill at 65° C.									
Shredded	53.0	52.2	0.8	29.6	22.6	43.3			
Shredded	53.0	52.5	0.5	29.6	22.9	43.6	5.2	22.0	5.5
Rolled Shredded	54.4	53.3	1.1	30.2	23.1	43.3			
Rolled Shredded	53.6	53.0	0.6	30.1	22.9	43.2	5.3	21.7	5.5
Water Extraction—Outside Extractor at 60-62° C.									
Shredded	55.0	54.3	0.7	30.2	24.1	44.4			
Shredded	55.3	54.5	0.8	30.0	24.5	44.9	5.3	21.6	5.4
Rolled Shredded	54.8	54.0	0.8	29.8	24.2	44.8			
Rolled Shredded	56.2	55.3	0.9	30.3	25.0	45.2	5.1	21.7	5.3
6.72 mm.	55.7	54.5	1.2	30.2	24.3	44.6			
6.72 mm.	56.3	55.3	1.0	30.2	25.1	45.4	5.3	20.8	5.3
4 mm.	55.7	54.3	1.4	30.1	24.2	44.6	4.8	21.2	5.4
50% Acetone-Water—Reed-Churchill at 60° C.									
Shredded	57.4	56.4	1.0	28.7	27.7	49.1			
Shredded	57.6	56.5	1.1	29.1	27.4	48.4	4.3	22.0	5.2
Rolled Shredded	57.6	56.4	1.2	28.8	27.6	48.9			
Rolled Shredded	57.4	56.2	1.2	28.7	27.5	48.9	4.5	21.5	5.2
6.72 mm.	58.7	57.4	1.3	29.3	28.1	49.0			
6.72 mm.	58.9	57.6	1.3	29.3	28.3	49.1	4.2	21.1	5.2
4 mm.	59.3	57.9	1.4	29.9	28.0	48.4			
4 mm.	59.0	57.5	1.5	29.4	28.1	48.8	4.1	21.3	5.2
2 mm.	59.4	57.8	1.6	29.2	28.6	49.5			
2 mm.	59.4	57.8	1.6	29.2	28.6	49.5	4.2	21.2	5.2
1 mm.	59.7	57.9	1.8	29.4	28.5	49.2			
1 mm.	59.6	57.8	1.8	29.3	28.5	49.3	4.2	21.3	5.2
60 mesh (0.25 mm.)	60.4	58.3	2.1	29.7	28.6	49.0			
60 mesh (0.25 mm.)	60.3	58.3	2.0	29.5	28.8	49.4	4.1	21.1	5.5
200 mesh (0.074 mm.)	60.7	58.6	2.1	29.7	28.9	49.3			
200 mesh (0.074 mm.)	60.7	58.7	2.0	30.3	28.4	48.4	4.1	21.3	5.5

*Purity is 100 times the tannin divided by the soluble extractives.

Volumes of approximately 750 ml. of extractives were collected in one-liter Erlenmeyer flasks in 6 hours. After the extraction was completed, the acetone was removed from the acetone-water extractives by distillation, and the aqueous residues containing the extracted matter were transferred to one-liter volumetric flask, made to volume, and analyzed in the usual way.

Some difficulty was experienced in extraction of the 60 mesh and 200 mesh samples with a 50 per cent acetone-water mixture. To minimize this and prevent fines from clogging the cotton mat, a layer of purified quartz sand about one-half inch deep was spread over the cotton on the bottom of the extraction tube before the canaigre was added.

Table V shows the data obtained in these studies.

Duplicate results are given to show the agreement obtained. The 50 per cent acetone-water extraction not only gave the most concordant results but also an increased efficiency of extraction. This was greater as the particle size became smaller and more uniform. The amount of tannin extracted reached a maximum with 2 mm. particle size and from that point on was practically constant to and including the 200 mesh size.

Difficulties in water extraction of canaigre were experienced with particle sizes smaller than the shredded material. This might be attributed to the amount of fines caused by grinding in a Wiley mill. Extraction of material ground to pass a 4 mm. screen was possible with the outside extractor but was not efficient because of clogging and subsequent slowness of extraction.

A study of the data in Table V shows that the values for tannin and total and soluble extractives were materially increased by extraction with a 50 per cent acetone-water mixture and the use of more finely and uniformly ground samples. Non tannin values were slightly higher with water extraction than with acetone-water extraction on samples ranging in state of subdivision from shreds to 6.72 mm. Also, somewhat higher values for reducing sugars were obtained by water extraction. These may be explained by the partial hydrolysis of sucrose. Total sugars agreed very well by all the methods of extraction. Since the particle size of 1 mm. obtained by grinding in the Wiley mill was convenient to prepare and since it gave maximum values for extraction, it was used in subsequent experimental work dealing with the effects of concentration of acetone, the time of extraction, and the volume of extractive.

Effect of Concentration of Acetone:

In studying the effects of concentration of acetone, mixtures containing 5, 10, 25, 50, and 75 per cent by volume of acetone in water were employed. The extraction was conducted for 6 hours at 60° C. in a Reed-Churchill extractor on a canaigre sample ground to pass a 1 mm. screen. The results are shown in Table VI.

It was not possible to extract the 1 mm. sample with 5 and 10 per cent acetone, and because of clogging and slowness of extraction an incomplete

TABLE VI

EFFECT OF CONCENTRATION OF ACETONE IN ACETONE-WATER MIXTURES ON EXTRACTION OF CANAIGRE*

(*Results on Moisture-Free Basis*)

Acetone	Volume of Extractives	Total Extractives	Soluble Extractives	Insolubles	Non Tannin	Tannin	Purity†
%	ml.	%	%	%	%	%	
5‡							
10‡							
25§	375	60.5	57.0	3.5	29.6	27.4	48.1
50	750	59.6	57.9	1.7	29.4	28.5	49.2
75	750	56.8	55.6	1.2	27.5	28.1	50.5

*Canaigre ground in a Wiley mill to pass a 1 mm. screen; extracted in a Reed-Churchill extractor at 60° C.
†Purity is 100 times the tannin divided by the soluble extractives.
‡Extraction impossible owing to swelling of starch and clogging.
§Inadequate collection because of clogging.

extraction was obtained with 25 per cent acetone. No difficulty, however, was experienced in the extraction with 50 per cent acetone, and this mixture gave the highest tannin value. Although the tannin extracted by 75 per cent acetone was nearly as high as that obtained with 50 per cent, the total and soluble extractives and non tannins were lower. There appears to be no advantage in extracting with 75 per cent acetone instead of 50 per cent.

Effects of Time and Volume of Extractive:

Table VII presents data on the effects of time of extraction and volume of extractive in the extraction of canaigre ground to pass a 1 mm. screen.

Extractions for 2, 3, 4, 5, and 6 hours, respectively, with 750 ml. of 50 per cent acetone showed no marked differences in total extractives and tannin. The 4, 5 and 6 hour extractions, however, indicated some slight trends toward higher values. Extractions for 6 hours in which 250, 500, 750, and 1000 ml., respectively, of extractive were collected gave about the same results indicating that the volume of extractive collected had little effect.

From these data it is evident that canaigre extractives are easily soluble in 50 per cent acetone at 60° C. Variations in time of extraction and volume of extractive, within the ranges studied, have no marked effect on the efficiency of extraction.

On the basis of these results, an extraction procedure was selected which appeared acceptable in regard to efficiency of extraction and adaptability to working day schedules. In this procedure, the extraction tubes were placed in a Reed-Churchill extractor and maintained at 60° C. throughout the extraction period. The 50 per cent acetone-water mixture was added at regular intervals and allowed to percolate through the sample. A volume of 500 ml. of extractive was collected in 5 hours.

Efficiency of Method:

To test the efficiency of this method a canaigre sample ground to pass a 1 mm. screen was extracted for one day, followed by the collection of an additional 500 ml. on a second day. The extractions and analyses were made in triplicate. The acetone-water mixture was heated to 60° C. before addition to the extraction tube. The tannin values obtained for the first day's extractions were 28.85, 28.92 and 28.79 per cent. Negative tests for tannin with gelatin-salt solution were obtained on the solutions from the second day's extractions. The soluble extractives in these solutions were 0.46, 0.45 and 0.41 per cent, respectively. They showed no tannin on analysis.

For further comparison of extraction methods, seven samples of canaigre roots harvested from an experimental plot at Lubbock, Texas and two samples collected in Arizona were extracted for analysis with water and 50 per cent acetone, respectively. The water extractions were made on sliced samples in a Reed-Churchill extractor at 65° C., one liter of extractives being collected in 7 hours. The 50 per cent acetone extractions were made on samples ground to pass a 1 mm. screen in a Reed-Churchill extractor at 60° C., 500 ml. of extractives being collected in 5 hours. The values obtained are shown in Table VIII.

The data show that the percentages of tannin obtained by extraction with 50 per cent acetone are from 3.6 to 6.7 higher than those obtained by the water extraction method. Since there were no marked changes in non tannin values, the higher extractives obtained were due almost entirely to more complete extraction of tannin. Continued extraction of these nine samples for a second day and analysis of the extractives showed that the tannin, soluble in 50 per cent acetone, had been completely removed.

TABLE VII

EFFECT OF TIME AND VOLUME OF EXTRACTIVES ON THE EXTRACTION OF CANAIGRE WITH 50 PER CENT ACETONE-WATER MIXTURE*

(Results on Moisture-Free Basis)

Time	Volume of Extractives	Total Extractives	Soluble Extractives	Insolubles	Non Tannin	Tannin	Purity†
Hours	ml.	%	%	%	%	%	
2	750	59.0	56.9	2.1	29.1	27.8	48.8
3	750	59.0	56.8	2.2	29.1	27.7	48.8
4	750	59.6	57.6	2.0	29.4	28.2	49.0
5	750	59.7	57.5	2.2	29.5	28.0	48.7
6	750	59.6	57.9	1.7	29.4	28.5	49.2
6	250	59.3	57.4	1.9	29.2	28.2	49.1
6	500	59.5	57.5	2.0	29.2	28.3	49.2
6	750	59.6	57.9	1.7	29.4	28.5	49.2
6	1000	59.5	57.8	1.7	29.2	28.6	49.5

*Canaigre ground in a Wiley mill to pass a 1 mm. screen; extracted in a Reed-Churchill extractor at 60° C.
†Purity is 100 times the tannin divided by the soluble extractives.

TABLE VIII

EXTRACTION WITH WATER AND WITH 50 PER CENT ACETONE-WATER MIXTURE OF CANAIGRE ROOTS HARVESTED AT LUBBOCK, TEXAS IN 1946

(Results on Moisture-Free Basis)

Samples	Total Extractives	Soluble Extractives	Insolubles	Non Tannin	Tannin	Purity†
	%	%	%	%	%	
			*Water Extraction at 65° C.**			
1	47.1	45.4	1.7	20.8	24.6	54.2
2	49.1	46.4	2.7	20.2	26.2	56.5
3	49.8	48.2	1.6	21.3	26.9	55.8
4	51.9	50.3	1.6	23.7	26.6	52.9
5	46.2	44.2	2.0	22.2	22.0	49.7
6	50.8	48.1	2.7	20.5	27.6	57.4
7	53.6	52.3	1.3	25.1	27.2	52.0
8	48.4	45.9	2.5	17.4	28.5	62.1
9	51.8	49.3	2.5	17.6	31.7	64.3
			50% Acetone-Water Extraction at 60° C.‡			
1	53.3	50.3	3.0	20.7	29.6	58.8
2	54.9	51.9	3.0	20.4	31.5	60.7
3	54.7	51.8	2.9	21.2	30.6	59.1
4	57.8	54.8	3.0	23.7	31.1	56.8
5	52.7	49.7	3.0	22.1	27.6	55.5
6	58.1	55.0	3.1	21.1	33.9	61.6
7	59.3	55.1	4.2	24.3	30.8	55.9
8	56.1	53.0	3.1	17.8	35.2	66.4
9	59.5	56.3	3.2	18.1	38.2	67.8

*Slices extracted in a Reed-Churchill extractor to 1 liter in 7 hours.

†Purity is 100 times the tannin divided by the soluble extractives.

‡Canaigre ground in a Wiley mill to pass a 1 mm. screen; extracted in a Reed-Churchill extractor to 500 ml. in 5 hours.

Summary and Conclusions

Methods for the extraction of canaigre for the determination of tannin were studied. Sliced and shredded samples were extracted with water (a) in Clarke-Frey extractors by the reflux method of the A.L.C.A., (b) in Reed-Churchill extractors at 65° C. and (c) in a modified outside extractor which permitted reflux extraction at 60° to 62° C. The Clarke-Frey extractors extracted more tannin, but the presence of starch produced slow-filtering solutions, which were unsatisfactory for analysis. The Reed-Churchill and outside extractors gave less complete extraction of tannin, but the solutions filtered satisfactorily. None of these water-extraction methods gave complete extraction of tannin.

In particle size studies, it was shown that samples of canaigre ground to pass a 4 mm. screen could be extracted with water in an outside extractor at 60° to 62° C. but not in the other extractors. When sliced samples were used in a Reed-Churchill extractor and temperatures of extraction and volumes of

extractives were varied, the best results were obtained at 60° and 65° C. with one-liter collections, but these results were lower than those obtained by other methods.

Comparative extractions of canaigre with water and with acetone-water mixtures showed 50 per cent acetone could be successfully used to extract tannin from finely ground samples in a Reed-Churchill extractor at 60° C. Concordant and maximum tannin values were obtained with samples prepared in particle sizes of 2 mm., 1 mm., 60 mesh (0.25 mm.), and 200 mesh (0.074 mm.). Coarser preparations gave less complete extraction.

Studies of the effects of concentration of acetone, time of extraction and volume of extractives indicated that canaigre samples ground to pass a 1 mm. screen could be extracted quantitatively with 500 ml. of 50 per cent acetone in a Reed-Churchill extractor at 60° C. in 5 hours.

When water extraction of nine samples of sliced canaigre was compared with 50 per cent acetone extraction of the same samples ground to pass a 1 mm. screen, the results indicated that 50 per cent acetone extracted the tannin quantitatively and gave concordant values ranging from 3.6 to 6.7 per cent higher than those obtained by water extraction.

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Discussion:

F. F. Marshall: We are again indebted to Mr. Luvisi and Mr. Rogers for this fifth paper in the series on canaigre. The paper is quite complete. It is stated in the paper that the need for a special analytical procedure was made necessary from the fact that greater yields were obtained in extract than was

indicated to be available in the raw material. I would like to ask Mr. Luvisi if the agreement between the analysis and production are in line when using the solvent extraction procedure?

F. P. LUVISI: Yields obtained by leaching canaigre roots with water and calculating this yield on the analytical value for tannin obtained by an acetone-water extraction did not give a leaching efficiency as high as 100 per cent. Leaching efficiencies of 100 per cent or over were obtained when the yields were calculated on the analytical tannin value obtained by water extraction.

MARSHALL: Grassmann and his group showed that the additional material extracted from pine and some other tanning materials with solvents showed rather high insoluble matter which upon bisulfiting produced a slack tanned piece of leather. Hence it was their conclusion that the additional material taken out by the solvents was of a different nature than the material that was removed by water alone. Have you made a study to determine if there is a difference in the material obtained from canaigre?

LUVISI: Our figures were not too much higher. The highest figure obtained was three and one-half per cent. But that was due to material clogging in the tube. However, some work was done at the Eastern Regional Laboratory which showed that some of the insolubles in an acetone-water extraction consist of chrysophanic acid. Other than that, I have no explanation.

MARSHALL: If I may digress a little from the present paper, which is presented for analytical procedure only, I note that the purity is somewhat lower in these analytical figures than that presented in the last paper on the extract basis. Is this simply due to a different type of raw material being extracted or would you obtain a lower purity through the use of the solvent?

LUVISI: We have been able to show that with an acetone-water mixture we can get higher purities on an analytical basis. Other than that, I don't get your point.

MARSHALL: The point was that in the last paper higher purities were obtained in extract on canaigre. The purity ran, I think, somewhere around 60 per cent.

LUVISI: I think Mr. Rogers might answer that.

J. S. ROGERS: There is quite a difference in the purities of different strains of canaigre and it is perfectly possible—that the ones that were reported in the previous paper to which you referred, came from a different strain of canaigre. The canaigre roots that grow in Arizona apparently run higher in tannin and have a higher purity than those that are native to New Mexico and Texas. In one of the previous papers we gave data on roots from both of those sources. In the last paper higher purities were obtained in both liquors and extracts by removal of sugars by fermentation.

H. B. MERRILL: I take it that you would expel the acetone from your extract before you make the analysis?

LUVISI: Yes.

L. SHEARD: Mr. Rogers, I understand that your laboratory is cultivating quite a quantity of canaigre roots under your direction. How long do you estimate it will be before you have enough to make a practical tannery test?

ROGERS: We do not know. We had hoped we might have in the next year enough canaigre to run some semi-commercial tanning tests, but we have not been able to get the large scale plantings of canaigre made. About three acres were planted rather late last fall in New Mexico by a cooperative organization. We are trying to harvest them this year. We hope to be able to expand the plantings this year so that by next summer we can have ten acres of roots to harvest.